
Contemporary Microscopy and Biophotonics

BioEn 498/599, Phys 427B

Problem Set #4

Due 5/11/2016

1. **Optical image processing.** A 4f optical system is shown in figure 1. A point source is a focal length from the first lens, a focal length from a photographic slide, a focal length away from a second lens, which is a focal length from a screen (or detector).
- (a) Start by replacing the slide with an iPhone screen (ignore the point source and lens A) and assume the light emitted by the phone is not coherent. What do you see on the image screen? (In a couple words. Hint: The lens is making an image at infinity.)
 - (b) Now consider the original device described in the question. What is the difference between the slide, illuminated by a point source and the cell phone screen?
 - (c) The Huygens-Fresnel Principle states that every point along a wave front acts as a secondary source. What is the phase difference between the red and the green secondary sources represented as points on the aperture plane shown in the figure? (The colors are used to differentiate the secondary sources. Assume light is monochromatic. Hint: I have drawn wave fronts from the two sources for you. You should be able to use geometrical arguments, based on these wave fronts to analyze the remaining questions.)
 - (d) What is the phase difference between the between the red and green rays when they strike the image screen at $x = 0$?
 - (e) Show the phase difference between the between the red and green rays is:

$$\Delta\phi(x|x') = -Kx', \quad (1)$$

where $K(x) \equiv kx/f_B$, when they strike the image screen at x ?

- (f) What is the intensity at the screen given a position-dependent transmittance of the slide $\rho(\vec{x}')$? Hint: Fix point x and integrate the wave amplitude over all sources x' on the screen. You should get the following equation for the wave amplitude:

$$\psi_{\text{tot}}(x) = \psi_0 e^{i\phi(x,t)} \int_{-\infty}^{+\infty} dx' \rho(x') e^{-iKx'}. \quad (2)$$

2. Derive the Point Spread Function and Diffraction Limit for a circular aperture.

As we discussed in class, a point source imaged through a diaphragm will result in a finite size image. The intensity profile of this image is called the *Point Spread Function*. Re-imagine figure 1 as a microscope with the photographic slide replaced by a circular aperture radius $a = D/2$, representing the finite numerical aperture of the objective.

(a) What is the aperture function $\rho(\vec{x}')$?

(b) Derive the total wave amplitude at the screen. Hint: Start with the equation:

$$\psi(\vec{x}, t) = \frac{\varepsilon_A e^{i(kR - \omega t)}}{R} \int_{\Sigma} d^2x' \exp\left(-i\vec{K} \cdot \vec{x}'\right), \quad (3)$$

where $\vec{K} \equiv k\vec{x}/f_B$. You will need to know the following definitions:

$$J_m(u) = \frac{i^{-m}}{2\pi} \int_0^{2\pi} d\nu \exp[i(m\nu + u \cos \nu)] \quad (4)$$

where J_m is a Bessel function of the first kind, order m . Another important property is:

$$\frac{d}{du} [u^m J_m(u)] = u^m J_{m-1}(u). \quad (5)$$

You should get the answer:

$$\psi(\vec{x}, t) = \pi a^2 \frac{\varepsilon_A e^{i(kR - \omega t)}}{R} \left[\frac{2J_1(u)}{u} \right]_{u=kra/f_B}, \quad (6)$$

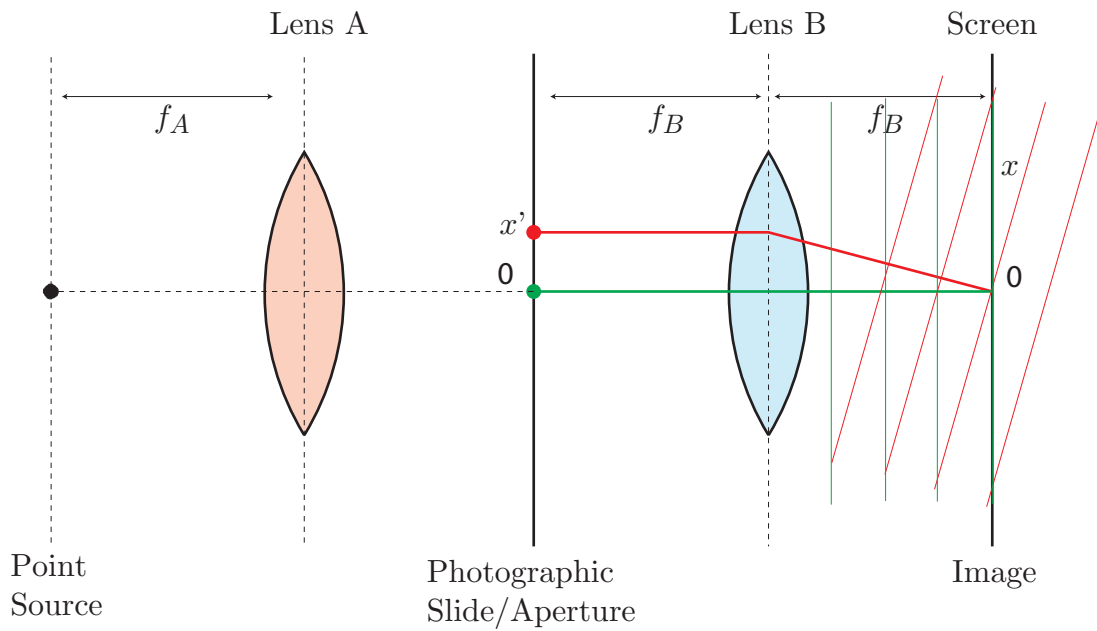
where $r \equiv |\vec{x}'|$.

(c) What is the intensity of the light from a point source? Please plot the intensity $I_{\text{PSF}}(\vec{x})$ as a function of the image coordinate \vec{x} along the x axis. This profile is known as an Airy disk and is the Point Spread Function for a circular aperture. (Hint: Ignore the pre-factors in eqn 6 and remember that $I \propto |\psi|^2$.)

(d) Argue that the image at the CCD is the convolution of the source density with the point spread function:

$$I_{\text{image}}(\vec{x}) \propto I_{\text{PSF}} \otimes i_{\text{object}}(\vec{x}) = \int_{\Sigma} d^2x' I_{\text{PSF}}(\vec{x} - \vec{x}') i_A(\vec{x}'), \quad (7)$$

where i_{object} is the areal source-intensity density or equivalently the intensity at the CCD in the absence of diffraction (assuming that the image is in perfect focus).



- (e) Find the position of the first zero of $J_1(u)/u$. Argue that the diffraction limit corresponds to a peak spacing corresponding to this zero. Show that the diffraction limit is:

$$\ell_{\min} = 1.22 \frac{\lambda_0 f_A}{nD} \approx 1.22 \frac{\lambda_0}{2NA}, \quad (8)$$

where n is the index of refraction. (Hint: Remember to use the magnification of the microscope to convert the image coordinates into object coordinates. Remember that the wavelength in a medium is shorter than the wavelength in vacuum λ_0 .)